

Preparation and Enantioseparation Property of Chiral Stationary Phases Based on Cellulose Tris(3,5-dimethylbenzoate) — A New Way to Prepare Polysaccharide-coating Type Chiral Stationary Phases*

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Abstract A new method to prepare polysaccharide-coating type chiral stationary phases (CSPs) was developed in this work. As a typical example, naked silica gel was coated by cellulose, which was then derivatized with 3,5-dimethylbenzoyl chloride to afford cellulose tris(3,5-dimethylbenzoate)-silica gel (CTDBS) complex. The silanols on CTDBS were end-capped with 3-aminopropyltriethoxysilane to obtain CSP 1. The amino groups on CSP 1 were further end-capped with 3,5-dimethylbenzoyl chloride to give CSP 2. The silanols on CTDBS were end-capped with methyltrimethoxysilane to yield CSP 3. CSPs 1–3 were characterized by FTIR, solid-state ¹³C-NMR and elemental analysis. The enantioseparation abilities of CSPs 1–3 were evaluated with structurally various chiral analytes. The enantioseparation results demonstrated that the end-capping moieties on CSPs 1 and 2 significantly affected enantioseparation. In addition, the effect of the structures of chiral analytes and end-capping moieties on the retention factors and the resolutions was discussed.

Keywords: Cellulose tris(3,5-dimethylbenzoate); Chiral recognition; Chiral stationary phase; High-performance liquid chromatography.

INTRODUCTION

Since 1980s, chiral high-performance liquid chromatography (HPLC) for separating the enantiomers of racemates has attracted increasing attention. This technique is usually employed to determine enantiomer contents, or to prepare pure enantiomers^[1–6]. Chiral stationary phases (CSPs) play an essential role for the enantioseparation by HPLC. In this field, the major works are concerning the development of various types of CSPs, which are desired to separate the enantiomers of a wide range of chiral compounds. Generally the well-known CSPs are prepared by coating polysaccharide derivatives on solid supports (mostly 3-aminopropyl silica gel)^[7–9], or by covalently immobilizing low-molecular-weight chiral molecules^[10–12], synthetic chiral polymers^[13, 14], or polysaccharide derivatives^[15] on functionalized silica gel. From the point of practicable application view, the methods to prepare CSPs by coating polysaccharide derivatives on supports are more preferable, because polysaccharides, such as cellulose, amylose and chitosan, *etc.* are readily available.

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Moreover, the CSPs can be easily prepared in bulk. Meanwhile polysaccharide type CSPs possess higher loading for enantiomers than other type CSPs^[6]. Accordingly, the chiral columns, which are packed with the CSPs derived from polysaccharide derivatives, are applied worldwide in pharmaceutical industry and in research. Okamoto *et al.* have published many works in this area^[16–18], and recently they still developed various methods to immobilize polysaccharide derivatives on silica gel in order to improve the CSPs stability^[19–21]. Among polysaccharides, cellulose was mostly employed to prepare CSPs^[22, 23]. In early stage, Okamoto and coworkers prepared cellulose tribenzoate and its derivatives with different substituents on the phenyl groups^[7]. Afterwards, the columns packed with the CSPs that were prepared by coating cellulose tribenzoate and tris(4-methylbenzoate) on silica gel were commercialized as CHIRALCEL OB and CHIRALCEL OJ. However, they found that cellulose tris(3,5-dimethylbenzoate) was insoluble in common organic solvents^[7]. Therefore, it could not be dissolved and coated on the silica gel. Methyl is an electron-donating group, and 3,5-dimethylphenyl is a π -base, which tends to interact with π -acid of chiral compounds. In addition, appropriate steric hindrance is necessary for chiral recognition. 3,5-Dimethylbenzoyl can appropriately enlarge the steric hindrance for the interaction between the chiral selector and analytes. This view is evidenced by cellulose tris(3,5-dimethylphenylcarbamate), which has been commercialized and became a widely applied chiral selector. Thus, cellulose tris(3,5-dimethylbenzoate) may be a satisfactory chiral selector for enantioseparation. The difficulties in coating cellulose tris(3,5-dimethylbenzoate) on silica gel addressed our investigations to alternative procedures, *i.e.* a polysaccharide was coated on silica gel, and then derivatized.

The silanols on silica gel were end-capped to afford a polysaccharide type CSP. As a typical model, cellulose was dissolved in a solution of LiCl in *N,N*-dimethylacetamide (DMAc), and was then coated on silica gel. The coating cellulose was modified with 3,5-dimethylbenzoyl chloride, followed by end-capping the silanols on the silica gel with various groups to afford new CSPs. The enantioseparation ability of these CSPs was preliminarily evaluated. The enantioseparation properties of the different CSPs were compared.

EXPERIMENTAL

Materials

Silica gel (SMB300) was purchased from Fuji Silysia Chemical Ltd. (Japan) with a particle size of 5 μm , a pore size of 30 nm, and a surface area of 90 m^2/g . Cotton linter pulp (α -cellulose) was available from Hubei Chemical Fiber Group Ltd. (Xiangfan, China), with a viscosity-average molecular weight (M_η) of 8.0×10^4 . Methyltrimethoxysilane (MTMS) was obtained from Alfa Aesar China (Tianjin, China) Co., Ltd. 3,5-Dimethylbenzoic acid was from Shanghai Zhuorui Chemical Co., Ltd (China). Pyridine was refluxed with CaH_2 and redistilled. Triethylamine (TEA) was dried over P_2O_5 and redistilled. Toluene was dried with sodium and redistilled. DMAc was dried over molecular sieves (0.4 nm) before use. All other chemicals used for the CSPs preparation were of analytical grade and used as received. All mobile phases were freshly prepared, filtered and degassed. Chiral analytes were available from Nhwa Pharmaceuticals Co., Ltd (Jiangsu, China), Huahai Pharmaceuticals Co., Ltd (Zhejiang, China), *etc.*, or were prepared according to the reported method^[24].

Instrumentation

IR spectra were recorded on a Nicolet FTIR instrument (USA) with KBr pellets. The thermogravimetric analysis (TGA) was conducted on a thermogravimetric analyzer (TGA Q50 V20.10 Build 36, USA). Elemental analysis (EA) was carried out with a VarioEL III CHNOS analyzer (Germany). Solid-state ^{13}C -NMR spectra were recorded on a Bruker spectrometer (AVANCE III 400WB, Switzerland), operating at 100 MHz. The CSPs were packed into stainless steel columns (250 mm $\times$ 4.6 mm) with a model of 1666 Alltech slurry packer (USA). Enantioseparation was performed on an Agilent 1100 chromatograph (USA), consisting of an Agilent G1365B DAD, an Agilent G1311A Quat Pump, an Agilent G1379A degasser and an Agilent G1313A ALS autosampler.

Preparation of the CSPs

Cellulose dissolution

Cotton linter pulp (0.70 g) was added in DMAc (25 mL), and the resulting mixture was heated to reflux for 1 h and stirred under a nitrogen atmosphere. Afterwards the mixture was cooled to ambient temperature, and the cellulose was collected by filtration washing three times with acetone. The cellulose was subsequently dried at 60 °C. LiCl (6.50 g) was dissolved in DMAc (65 mL) at 100 °C. To this solution, the dried cellulose was added, and the mixture was stirred at 80 °C for 3 h to give a clear solution.

Coating silica gel with cellulose

Silica gel (10.30 g) was mixed with the prepared cellulose solution (51.30 g). DMAc was removed under vacuum on a rotation evaporator. The residue was washed with water, and cellulose-coated silica gel as a white powder (10.78 g) was obtained after drying in vacuum. IR (KBr, cm^{-1}): 3740–3119 (—OH), 2909 (C—H), 1103 (Si—O); EA (%): C 2.47, H 0.93.

Derivatization of the cellulose coated on silica gel

3,5-Dimethylbenzoyl chloride was prepared according to the classic method with 3,5-dimethylbenzoic acid and thionyl chloride. 3,5-Dimethylbenzoic acid (9.40 g, 62.60 mmol) was placed in a 100 mL flask, in which thionyl chloride (35 mL) was added dropwise at 25 °C. Then the solution was heated to reflux for 3 h. The excess thionyl chloride was distilled in vacuum to give a pale yellow liquid, *i.e.* 3,5-dimethylbenzoyl chloride. Cellulose-coated silica gel (9.70 g), TEA (5 mL) and DMAP (0.40 g) were mixed in pyridine (150 mL), and then 3,5-dimethylbenzoyl chloride (10.10 g) was slowly added. The resulting mixture was stirred at ambient temperature for 24 h, and then at 80 °C for 12 h. After the completion of the reaction, the solid was collected by filtration and washed three times with diethyl ether. Cellulose tris(3,5-dimethylbenzoate)-silica gel (CTDBS) (10.81 g) was a grey white powder after the removal of the volatiles. IR (KBr, cm^{-1}): 2959 (C—H), 1737 (—CO₂—), 1317 (C—O), 1109 (Si—O); Solid-state ¹³C-NMR (25 °C, δ): 203–183, 170–164 (—CO₂—), 143–124 (Ar C), 108–97 (C-1), 85–57 (C-2, C-3, C-4, C-5, C-6), 25–17 (Ar—CH₃); EA (%): C 11.66, H 1.80.

Preparation of CSP 1

CTDBS (6.86 g), 3-aminopropyltriethoxysilane (APTES, 10 mL) and TEA (3 mL) were mixed in toluene (75 mL). The mixture was stirred at 85 °C for 15 h. After the completion of the reaction, the solid was filtered and thoroughly washed with THF. CSP 1 (7.00 g) was a yellow powder after the removal of the volatiles. IR (KBr, cm^{-1}): 2945 (—CH₂—), 1734 (—CO₂—), 1312 (C—O), 1103 (Si—O); Solid-state ¹³C-NMR (25 °C, δ): 202–185, 168–163 (—CO₂—), 142–122 (Ar C), 107–96 (C-1), 83–56 (C-2, C-3, C-4, C-5, C-6, Si—O—CH₂CH₃), 46–38 (SiCH₂CH₂CH₂NH₂), 25–14 (Ar—CH₃, SiOCH₂CH₃, SiCH₂CH₂CH₂NH₂), 12–6 (SiCH₂CH₂CH₂NH₂); EA (%): C 11.68, H 1.64, N 0.40.

Preparation of CSP 2

CSP 1 (3.80 g), DMAP (0.45 g) and TEA (3 mL) were added in pyridine (65 mL), in which freshly prepared 3,5-dimethylbenzoyl chloride (3.30 g) was added dropwise. The mixture was stirred at 60 °C for 18 h. After being cooled to ambient temperature, the mixture was poured in diethyl ether. The precipitant was filtered and washed with diethyl ether till the filtrate became colorless. CSP 2 (3.81 g) was a brown powder after the removal of the volatiles. IR (KBr, cm^{-1}): 2949 (—CH₂—), 1736 (—CO₂—), 1316 (C—O), 1105 (Si—O); Solid-state ¹³C-NMR (25 °C, δ): 203–185, 168–164 (—CO₂—), 141–122 (Ar C), 107–97 (C-1), 83–57 (C-2, C-3, C-4, C-5, C-6, Si—O—CH₂CH₃), 47–36 (SiCH₂CH₂CH₂NH₂), 28–16 (Ar—CH₃, SiOCH₂CH₃, SiCH₂CH₂CH₂NH₂), 12–6 (SiCH₂CH₂CH₂NH₂); EA (%): C 15.88, H 1.99, N 0.36.

Preparation of CSP 3

CTDBS (3.04 g) was added in a solution of TEA (5 mL) and MTMS (5 mL) in toluene (50 mL). The mixture was stirred at 80 °C for 20 h. After being cooled to ambient temperature, the solid was collected by sucking filtration, and was then washed thoroughly with THF. CSP 3 (3.00 g) as a yellow powder was obtained after the

removal of the volatiles in vacuum. IR (KBr, cm^{-1}): 2967 ($-\text{CH}_2-$), 1737 ($-\text{CO}_2-$), 1314 (C—O), 1102 (Si—O); Solid-state ^{13}C -NMR (25 $^{\circ}\text{C}$, δ): 203–185, 168–165 ($-\text{CO}_2-$), 143–124 (Ar C), 107–95 (C-1), 84–57 (C-2, C-3, C-4, C-5, C-6), 53–47 (Si—O— CH_3), 26–15 (Ar— CH_3), –3.7 (Si CH_3); EA (%): C 11.14, H 1.83.

Column packing and chromatographic conditions

CSPs **1–3** were suspended in the mixture of isopropanol/*n*-hexane (90/10, *V/V*), and then sonicated for 7 min to form slurries, respectively. The slurries were poured into the chamber of the packing apparatus, and were pushed into the empty HPLC columns with *n*-hexane as the displacer solvent, under a pressure of 37.9 MPa. The packed columns were flushed with isopropanol/*n*-hexane (10/90, *V/V*) at a flow rate of 1 mL/min till a stable column pressure was achieved. Under this condition, the column efficiency was measured with biphenyl as the sample. The chiral analytes solutions (1 mg/mL) were prepared by dissolving the chiral analytes in ethanol and were filtered through 0.2 μm membrane. The injection volume was 10 μL . All mobile phases were filtered and degassed before use. During the enantioseparation, the flow rate was set at 1 mL/min, and the column temperature was set at 25 $^{\circ}\text{C}$.

RESULTS AND DISCUSSION

Preparation and Characterization of CSPs

The preparation schemes of CSPs **1–3** are shown in Fig. 1. The coating cellulose was heterogeneously derivatized with 3,5-dimethylbenzoyl chloride. Pyridine was used as the solvent for the derivatization reaction because the derivative, cellulose tris(3,5-dimethylbenzoate), is almost insoluble in pyridine. If other derivatives of cellulose are soluble in pyridine, the solvents, such as toluene or a mixture of pyridine and toluene can be used. CTDBS and CSPs **1–3** were characterized by IR spectra, in which the absorbance approximately at 1730 cm^{-1} corresponds to the esters in the derivatized cellulose, revealing the hydroxyls in the cellulose were successfully derivatized. The cellulose-coated silica gel and CTDBS were characterized by thermogravimetric analysis (Fig. 2).

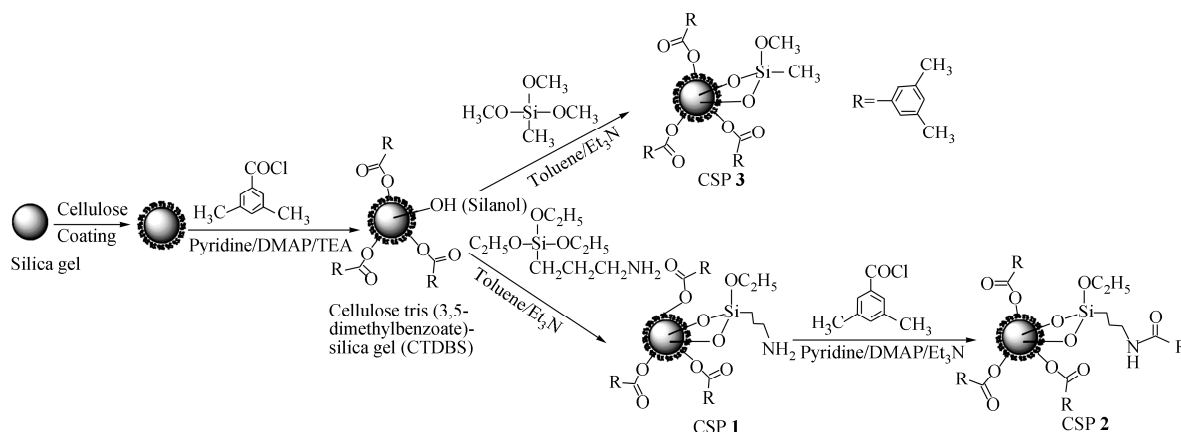


Fig. 1 The preparation scheme of CSPs **1–3**

From Fig. 2, the capacities of cellulose and cellulose tris(3,5-dimethylbenzoate) on silica gel were estimated as 4.8% and 14.0%, respectively. The degree of the substitution (DS) of hydroxyls on the coating cellulose was estimated as 95%. Therefore, the most hydroxyls in the cellulose that was coated on silica gel were modified. The silanols on CTDBS were end-capped with APTES to afford CSP **1**. The amino groups on CSP **1** were further end-capped by 3,5-dimethylbenzoyl giving CSP **2**. Comparing the solid-state ^{13}C -NMR spectra of CTDBS and CSP **1** (Fig. 3), the peaks at $\delta = 43$ and 10 correspond to the $-\text{CH}_2\text{NH}_2$ and SiCH_2- , indicating that the silanols were end-capped by 3-aminopropylsilyl. Similarly, in solid-state ^{13}C -NMR spectrum of CSP **3**, the peak at $\delta = 3.7$ corresponds to $\text{Si}-\text{CH}_3$, evidencing the silanols on CSP **3** were end-capped by methylsilyl.

The carbon content of CSP 2 was higher than that of CSP 1; while nitrogen content of CSP 2 was lower than that of CSP 1. This two results confirm that the amino groups on CSP 2 were end-capped by 3,5-dimethylbenzoyl.

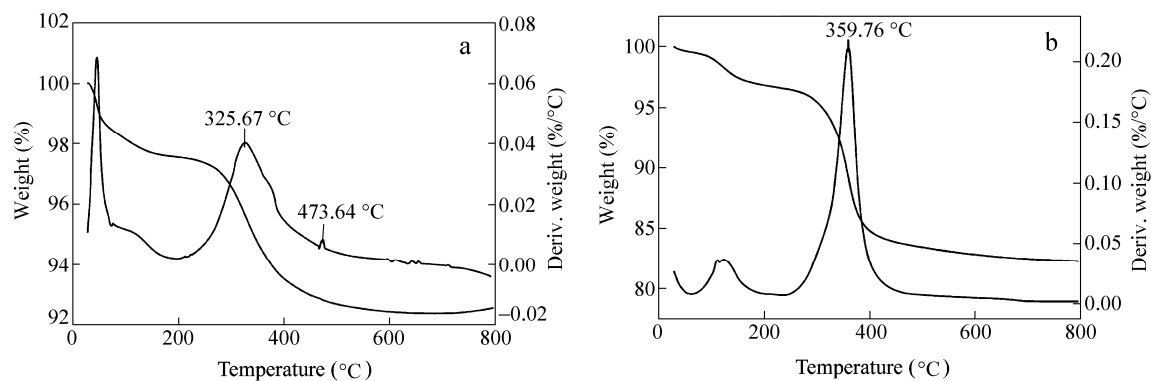


Fig. 2 The thermogravimetric analysis of cellulose-coated silica gel (a) and CTDBS (b)

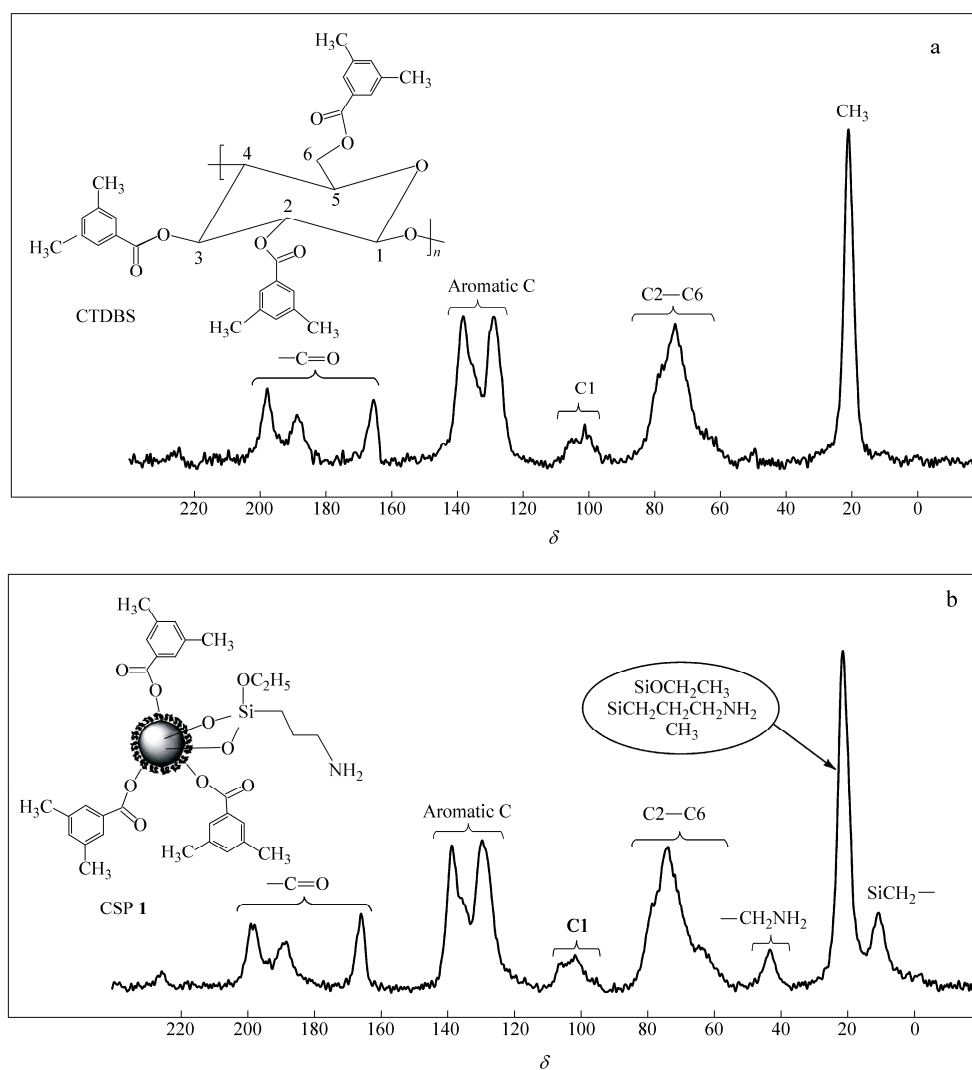


Fig. 3 ¹³C-NMR spectra of CTDBS (a) and CSP 1 (b)

The Evaluation in Enantioseparation Ability of CSPs 1–3

The efficiencies of the columns packed with CSPs 1–3 were determined as 13900, 11000 and 18500 plates per meter, respectively. The enantioseparation abilities of CSPs 1–3 were evaluated by using structurally various chiral analytes (Fig. 4) under the same mobile phase conditions of normal and reversed phases. The separation conditions for each individual chiral analyte were not optimized. Therefore, some analytes were not baseline separated. However, the enantioseparation abilities of CSPs 1–3 are still comparable under the same separation conditions. The enantioseparation parameters are listed in Table 1. Both CSPs 1 and 2 provided the enantioseparation of 10 compounds, while CSP 3 separated 12 compounds.

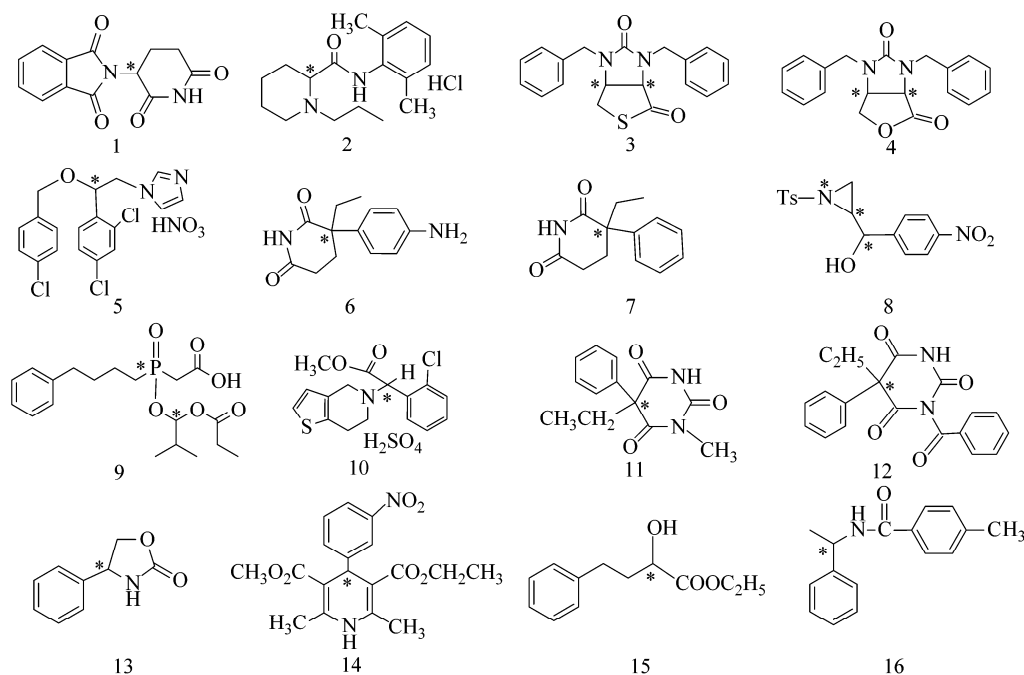


Fig. 4 The structures of chiral analytes resolved by CSPs 1–3

In view of the number of the separated compounds, the chiral recognition ability of CSP 3 is slightly better than that of CSPs 1 and 2. In addition, identical chiral analytes were not all separated by CSPs 1–3. The enantiomers of compounds 1 and 12, for example, were chirally recognized by CSP 1, but not by CSPs 2 and 3; compound 9 was separated by CSP 2, but not by CSPs 1 and 3.

The precursors of CSPs 1–3 are CTDBS, which were prepared in the same batch. Since CSPs 1–3 only differ in the end-capping groups of the silanols, obviously, the end-capping moieties significantly affect chiral recognition. Pirkle and coworker once discussed the influence of end-capping groups on the enantioselectivity of a chiral stationary phase^[25]. Just as a chiral recognition mechanism, the influence of end-capping groups on enantioseparation is very complicated and is difficultly substantiated. However, it is clear that the functional groups in the end-capping moieties of CSPs 1 and 2 were involved in the enantioseparation of chiral analytes.

In enantioseparation, it is believed that a chiral selector interact with two enantiomers of a chiral analyte to form two diastereoisomers. The difference in the stability of the two diastereoisomers causes enantioseparation^[26, 27]. For CSP 1, the silanols were end-capped by 3-aminopropylsilyl. The amines can interact with functional groups in chiral analytes forming H-bond *etc.* During chiral recognition, the amines in CSP 1 may be involved in the formation of the diastereoisomers. The enantiomers of compounds 1 and 12, for example, were chirally recognized by CSP 1, but not by CSPs 2 and 3. Besides interacting with cellulose tris(3,5-dimethylbenzoate), compounds 1 and 12 may also interact with the amines on CSP 1 resulting in the diastereoisomers formation. If the diastereoisomers are not equally stable, the two enantiomers are separated. In

this case, the amines on CSP **1** contribute the enantioseparation. On the contrary, if the diastereoisomers are equally stable, the enantioseparation does not take place. This inference is consistent with the fact that compounds 10, 11 and 14 were chirally separated by CSPs **2** and **3**, but not by CSP **1**.

Table 1. The chromatographic data of chiral compounds resolved by CSPs **1–3**

S/N	CSP 1				CSP 2				CSP 3			
	k_1	α	R_s	Eluent	k_1	α	R_s	Eluent	k_1	α	R_s	Eluent
1	4.12	1.17	1.30	A (30/70)	no separation				no separation			
2	no separation				no separation				0.22	1.30	0.45	D (45/45/10)
3	1.32	1.22	0.79	B (30/70)	5.00	1.16	0.88	A (10/90)	3.69	1.15	1.12	A (10/90)
4	1.30	1.36	1.88	C (50/50)	2.43	1.23	1.29	E (30/70)	1.73	1.33	1.75	E (30/70)
5	2.04	1.04	0.18	B (10/90)	2.42	3.41	1.02	B (10/90)	no separation			
6	3.50	1.19	0.79	B (30/70)	4.43	1.13	0.55	B (30/70)	1.46	1.06	0.55	E (50/50)
7	6.70	1.55	0.80	A (10/90)	7.54	1.39	1.73	E (10/90)	2.24	1.07	0.53	A (30/70)
8	1.91	1.74	3.94	D (15/15/70)	2.78	2.19	2.60	B (30/70)	0.67	1.60	3.02	B (90/10)
9	no separation				1.88	2.04	1.74	D (35/35/30)	no separation			
10	no separation				0.89	1.18	0.61	B (30/70)	0.73	1.07	0.77	B (30/70)
11	no separation				0.81	1.05	0.40	A (90/10)	0.70	1.05	0.50	A (90/10)
12	1.60	1.73	2.00	A (30/70)	no separation				no separation			
13	4.03	1.18	0.70	B (10/90)	no separation				0.91	1.26	0.29	F (60/40)
14	no separation				0.43	1.30	1.04	D (25/25/50)	0.34	1.52	0.86	A (70/30)
15	no separation				no separation				0.32	1.02	0.20	A (50/50)
16	2.13	1.10	0.43	C (10/90)	no separation				4.65	1.10	0.77	F (60/40)

The retention factors (k_1 and k_2) were calculated from the formulas: $(t_1 - t_0)/t_0$ and $(t_2 - t_0)/t_0$, where t_1 and t_2 are, respectively, the retention time of the first and the second-eluted enantiomer, and t_0 was determined by measuring the retention time of NaNO₂ solution. The separation factor (α) was calculated from the formula: k_2/k_1 . The resolution (R_s) was calculated from the formula: $2(t_2 - t_1)/(w_1 + w_2)$, where w_1 and w_2 are the bandwidth of the first and the second-eluted enantiomer, respectively.

Eluent: A: Ethanol/*n*-hexane; B: Isopropanol/*n*-hexane; C: Isobutyl alcohol/*n*-hexane; D: Methanol/ethanol/*n*-hexane; E: *n*-Propanol/*n*-hexane; F: Methanol/water.

As for CSP **2**, the amines were end-capped by 3,5-dimethylbenzoyls forming amides, which can also interact with chiral analytes through H-bonding. Besides, there are π - π interactions between 3,5-dimethylphenyl and other phenyls in chiral analytes. Similar to the behavior of the amines in CSP **1**, when 3,5-dimethylphenyls participate the formation of two differently stable diastereoisomers, the related chiral analytes can be enantiomerically separated. Compound 9 was separated by CSP **2**, but not by CSPs **1** and **3**, reflecting that 3,5-dimethylphenyls contribute to the enantioseparation. If the temporary diastereoisomers formed with the participation of 3,5-dimethylphenyls are equally stable, the corresponding compounds are not separated. On the other hand, the large end-capping moiety in CSP **2** may result in steric hindrance that prevents chiral analytes to access the functional groups in cellulose tris(3,5-dimethylbenzoate) and as a result, enantioseparation cannot take place. These two circumstances that cause no enantioseparation conform to the separation results of compounds 13 and 16, which were separated by CSPs **1** and **3**, but not by CSP **2**.

The silanols on CSP **3** were end-capped by methylsilyl, which is inactive to chiral analytes. In addition, methylsilyl is a small group that does not cause severe steric hindrance for chiral analytes to access the chiral selectors. Thus, the enantioseparation on CSP **3** is only owed to the function of cellulose tris(3,5-dimethylbenzoate).

Effect of the Structures of Chiral Analytes and the CSPs on the Retention and Resolution

Table 2 summarizes the enantioseparation results of chiral analytes 3, 4, 6 and 7 resolved by CSPs **1–3** under the same mobile phase conditions, which are the normal phase modes consisting of alcohols and *n*-hexane. In most cases, the retention factors on CSP **2** are bigger than those on CSPs **1** and **3** (Table 2). There are amides and 3,5-dimethylphenyls in the end-capping moieties in CSP **2**, rather than only amines in CSP **1**. More interactions may be formed between these two groups and the analytes, thus causing bigger retention factors on CSP **2**. When the

mobile phases contained the alcohols of lower content, such as 30% ethanol or isobutanol, the retention factors of compounds 6 and 7 on CSP 1 are bigger than those on CSPs 2 and 3. There are two exceptions, *i.e.*, when IPA was used as the mobile phase composition, the retention factors of compounds 6 and 7 on CSP 3 did not decrease as IPA content increased; and the retention factors of compound 7 on CSP 2 were the lowest when the mobile phases consisted of 50% or 70% isobutanol. IPA and isobutanol are both secondary alcohols. The structures of alcohols may affect the interactions between the mobile phases and the analytes, and further affect the retention factors of the analytes.

Table 2. The enantioseparation of chiral analytes 3, 4, 6 and 7 on CSPs 1–3 under the same mobile phase conditions

Chiral analytes	CSP 1			CSP 2			CSP 3			Eluent (V/V)
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	
3	0.78	1.22	0.59	1.45	1.10	0.66	1.16	1.09	0.86	EtOH/ <i>n</i> -hexane: 30/70
	0.28	1.29	0.30	0.87	1.07	0.60	0.74	1.06	0.67	EtOH/ <i>n</i> -hexane: 50/50
	0.16	1.35	0.16	0.66	1.06	0.53	0.59	1.05	0.54	EtOH/ <i>n</i> -hexane: 70/30
	0.67	1.24	0.39	1.43	1.10	0.60	1.09	1.16	0.75	IPA/ <i>n</i> -hexane: 50/50
	0.49	1.22	0.32	1.05	1.08	0.55	0.83	1.07	0.62	IPA/ <i>n</i> -hexane: 70/30
	1.39	1.19	0.58	2.44	1.11	0.60	1.76	1.11	0.77	Isobutanol/ <i>n</i> -hexane: 30/70
	0.87	1.19	0.54	1.03	1.08	0.57	1.03	1.09	0.59	Isobutanol/ <i>n</i> -hexane: 50/50
	0.72	1.17	0.39	0.89	1.06	0.48	0.78	1.06	0.55	Isobutanol/ <i>n</i> -hexane: 70/30
	0.89	1.20	0.78	1.77	1.11	0.60	1.31	1.10	0.84	Propanol/ <i>n</i> -hexane: 30/70
	0.37	1.19	0.43	0.99	1.08	0.52	0.79	1.07	0.64	Propanol/ <i>n</i> -hexane: 50/50
	0.24	1.27	0.39	1.29	1.16	0.48	0.61	1.05	0.50	Propanol/ <i>n</i> -hexane: 70/30
4	1.07	1.34	0.47	1.79	1.33	1.33	1.42	1.30	1.71	EtOH/ <i>n</i> -hexane: 30/70
	0.36	1.48	0.46	0.99	1.29	1.06	0.83	1.27	1.27	EtOH/ <i>n</i> -hexane: 50/50
	0.19	1.59	0.28	0.69	1.27	0.91	0.62	1.23	0.98	EtOH/ <i>n</i> -hexane: 70/30
	0.13	1.54	0.30	0.58	1.21	0.62	0.54	1.18	0.71	EtOH/ <i>n</i> -hexane: 90/10
	1.96	1.35	1.28	3.55	1.33	1.09	2.37	1.34	1.44	IPA/ <i>n</i> -hexane: 30/70
	0.91	1.39	0.98	1.73	1.32	1.02	1.28	1.30	1.23	IPA/ <i>n</i> -hexane: 50/50
	0.61	1.38	0.86	1.15	1.29	0.92	0.89	1.26	1.20	IPA/ <i>n</i> -hexane: 70/30
	0.74	1.27	0.61	0.94	1.26	0.77	0.77	1.22	0.92	IPA/ <i>n</i> -hexane: 90/10
	5.80	1.11	0.61	2.69	1.09	0.49	2.25	1.07	0.53	EtOH/ <i>n</i> -hexane: 30/70
	0.75	1.18	0.40	1.26	1.07	0.45	1.12	1.05	0.47	EtOH/ <i>n</i> -hexane: 50/50
6	0.33	1.23	0.21	0.73	1.05	0.43	0.70	1.04	0.44	EtOH/ <i>n</i> -hexane: 70/30
	0.18	1.24	0.10	0.55	1.04	0.34	0.54	1.03	0.36	EtOH/ <i>n</i> -hexane: 90/10
	1.66	1.21	0.74	2.06	1.10	0.50	0.97	1.05	0.45	IPA/ <i>n</i> -hexane: 50/50
	0.97	1.22	0.57	1.28	1.07	0.42	1.09	1.06	0.47	IPA/ <i>n</i> -hexane: 70/30
7	6.26	1.11	0.60	2.71	1.10	0.53	2.24	1.07	0.53	EtOH/ <i>n</i> -hexane: 30/70
	0.75	1.17	0.40	1.27	1.07	0.47	0.88	1.05	0.48	EtOH/ <i>n</i> -hexane: 50/50
	0.35	1.21	0.21	0.74	1.06	0.44	0.70	1.04	0.42	EtOH/ <i>n</i> -hexane: 70/30
	0.18	1.15	0.10	0.60	1.01	0.11	0.54	1.03	0.35	EtOH/ <i>n</i> -hexane: 90/10
	1.67	1.21	0.74	2.10	1.10	0.51	0.86	1.05	0.46	IPA/ <i>n</i> -hexane: 50/50
	0.93	1.21	0.58	1.29	1.08	0.48	1.09	1.06	0.48	IPA/ <i>n</i> -hexane: 70/30
	4.50	1.14	0.48	5.18	1.10	0.45	3.96	1.09	0.51	Isobutanol/ <i>n</i> -hexane: 30/70
	2.50	1.14	0.43	1.29	1.07	0.45	1.98	1.06	0.37	Isobutanol/ <i>n</i> -hexane: 50/50
	1.60	1.13	0.40	0.95	1.05	0.36	1.27	1.05	0.38	Isobutanol/ <i>n</i> -hexane: 70/30
	2.90	1.16	0.62	3.85	1.12	0.55	2.98	1.08	0.51	Propanol/ <i>n</i> -hexane: 30/70
7	1.16	1.15	0.49	1.80	1.09	0.51	1.46	1.06	0.40	Propanol/ <i>n</i> -hexane: 50/50
	0.64	1.13	0.32	1.05	1.06	0.36	0.90	1.04	0.27	Propanol/ <i>n</i> -hexane: 70/30

CONCLUSIONS

To improve the workability to coat insoluble derivatives of polysaccharides on solid supports, in the present work, cellulose was coated on silica gel, and the coating cellulose was then derivatized with 3,5-dimethylbenzoyl chloride. The silanols on CTDBS were end-capped by various groups giving new CSPs. This method is applicable to prepare polysaccharide-coating type CSPs. The end-capping groups of 3-aminopropylsilyl and 3,5-dimethylbenzoyl greatly affected the enantioseparation of chiral analytes. This feature may provide considerations to prepare new CSPs that are desired to separate a class of chiral compounds.

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